sustainability

Article

Phytochemical Characterization of *Callistemon lanceolatus* Leaf Essential Oils and Their Application as Sustainable Stored Grain Protectants against Major Storage Insect Pests

Thachappilly A. Ankitha [1], Naduvilthara U. Visakh [1,*], Berin Pathrose [1,*], Nicola Mori [2], Rowida S. Baeshen [3] and Rady Shawer [4]

[1] Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Thrissur 680656, Kerala, India
[2] Department of Biotechnology, Verona University, 37114 Verona, Italy
[3] Department of Biology, Faculty of Science, Tabuk University, Tabuk 71421, Saudi Arabia
[4] Department of Plant Protection, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria 21531, Egypt
* Correspondence: visakhnu17palazhi@gmail.com or visakh-2019-21-001@student.kau.in (N.U.V.); berin.pathrose@kau.in (B.P.)

Abstract: Food storage has been important since the dawn of agriculture and human settlement. Insect pests cause major losses to food grains during storage and production. Essential oils are good alternatives for chemical insecticides for the management of storage pests. Red bottlebrush, *Callistemon lanceolatus,* is a flowering plant of the Myrtaceae family. This research work aimed to extract the oil from bottlebrush leaves, and chemically characterize and assess their repellent and insecticidal properties against the cowpea seed beetle, *Callasobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae), and red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae), for the first time. The essential oil yielded by hydro-distillation of bottlebrush leaves was 1.02 ± 0.01%. GC-MS analysis determined the chemical composition of the volatile oil comprised 1,8-cineole (19.17%), α-terpineol (11.51%), α-pinene (10.28%), and α-Phellandrene (9.55%). The *C. lanceolatus* leaf oil showed potent repellence, contact toxicity, and fumigation toxic effects. In the contact toxicity assay, at 24 h, the LC_{50} values were 1.35, 0.52, and 0.58 mg/cm^2 for the red flour beetle, cigarette beetle, and cowpea seed beetle, respectively. Likewise, in the fumigation assay observed after 24 h, LC_{50} values of 22.60, 5.48, and 1.43 µL/L air were demonstrated for the red flour beetle, cigarette beetle, and cowpea seed beetle, respectively. Additionally, there was no significance found by a phytotoxicity assay when the paddy seeds were exposed to *C. lanceolatus* oil. The results show that the volatile oils from red bottlebrush leaves have the potential to be applied as a biopesticide. Therefore, *C. lanceolatus* leaf oil can be utilized as a bio-insecticide to control stored product insects.

Keywords: volatile essential oils; insecticidal activity; GC-MS; cigarette beetle; phytotoxicity; bottlebrush; sustainable agriculture

Citation: Ankitha, T.A.; Visakh, N.U.; Pathrose, B.; Mori, N.; Baeshen, R.S.; Shawer, R. Phytochemical Characterization of *Callistemon lanceolatus* Leaf Essential Oils and Their Application as Sustainable Stored Grain Protectants against Major Storage Insect Pests. *Sustainability* **2024**, *16*, 1055. https://doi.org/10.3390/su16031055

Academic Editor: Helvi Heinonen-Tanski

Received: 11 December 2023
Revised: 23 January 2024
Accepted: 23 January 2024
Published: 25 January 2024

1. Introduction

As the world's population is projected to reach 9 million by 2050, the quest for food is gaining predominant importance in the world. Ensuring food safety and quality despite the effects of pests in stored products is a major challenge faced by many countries. A major proportion of food losses in storage facilities and food processing occur because of stored product pests and their poor management [1]. Stored grain pests usually consume grains, bore into the kernel to deteriorate the germ portion, give out heat to destroy the stored grain products, and contaminate the product with their excretory products [2]. This results in significant losses, primarily because of nutritional depletion and decreased market value [2]. An estimated 70% of insect pests are thought to be a major risk to food that has been stored [3]. Thus, effective pest control in stored products is essential to minimize food losses, underscoring the broader importance of food safety and security within a resilient food system.

The red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae), is a predominant pest affecting stored goods in tropical countries [4]. Its significance stems from its rapid spread once established, its ability to locate and infest goods, and its capacity to infect a wide range of potential hosts [5]. The cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) is known to be a significant pest of wheat, sorghum, and maize, primarily found in tropical and subtropical regions [6]. It is considered the most harmful insect pest to both raw and processed tobacco, as well as a wide variety of other materials derived from both plants and animals [6]. Cigarette beetles are the minor pest of oil seeds, sage and dried fruit, cereals, flour, oil–cake, and various animal goods. The cowpea seed beetle, or *Callasobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), is a species of beetle that exploits stored products [7]. The massive quantitative and qualitative losses caused by these pests limit the level of utility and render the seeds unfit for either sowing or human consumption [8]. The persistent application of synthetic organic pesticides has led to an increase in insect and other arthropod pests' resistance [9].

Several synthetic insecticides have been banned or restricted due to their toxic effects, prompting a shift toward natural, less toxic, and environmentally friendly alternatives [10]. Natural insecticides are often preferred because they are selective, less persistent, have little or no resistance development, and degrade in the environment over a relatively short period [11]. Recent research has emphasized the significance of bio-pesticides as promising alternatives to synthetic pesticides for managing storage pests in crops [9]. Plant volatile organic compounds and plant essential oils are the most widely used biocides that have shown significant efficacy against pests and microbes [12–14]. In addition to their potential effects on growth rate, lifespan, and reproduction, these substances can also be used as fumigants, contact insecticides, repellents, and antifeedants [14].

The Myrtaceae family comprises more than 5800 species, which are renowned for their aromatic leaves that frequently contain essential oils, as well as their culinary, medicinal, and aesthetic qualities. The high terpene content of Myrtaceae plants is well known, and it occurs in both the shoots and the leaves [15]. The majority of their chemical components are obtained by hydro-distilling aerial parts to extract leaf oil. Essential oils from Myrtaceae typically contain sesquiterpene combinations with trace amounts of monoterpenes, which contribute to their bio-activity [15], making them potential biocides. The *Callistemon* genus, comprising shrubs and small trees, is commonly referred to as bottle brush due to its unique flower spikes resembling cylindrical brushes [16]. The red bottlebrush tree, *Callistemon lanceolatus* (Myrtales: Myrtaceae), is a native of Queensland [17]. Numerous biological activities, such as antioxidant, anti-diabetic, antimicrobial, and anti-proliferative have been reported for the aerial organs of *C. lanceolatus* [18–20].

Previous research has demonstrated that *C. lanceolatus* leaf essential oils have various biological activities such as antioxidant and anticancer activity [21], pharmacognostic and anti-inflammatory [22], anti-hyperglycemic, and antimicrobial properties [19]. The primary component 1,8-cineole, which is present in *C. lanceolatus*, showed significant antimicrobial activity on aflatoxin synthesis [23]. Some studies have shown the antibacterial activity of *C. lanceolatus* leaf essential oils against phytopathogenic bacteria and human pathogens, such as bacterial wilt of potato, *Ralstonia solanacearum* (Burkholderiales: Burkholderiaceae); angular leaf spot of cotton, *Xanthomonas axonopodis pv. Malvacearum* (Xanthomona-dales: Xanthomonadaceae); bacterial leaf spot on pepper plants, *Xanthomonas cam-pestris pv. Vesicatoria* (Xanthomonadales: Xanthomonadaceae); and rice leaf blight, *Xanthomonas oryzae pv. Oryzae* (Xanthomonadales: Xanthomonadaceae) [24], as well as antioxidant and anti-fungal activity, and aflatoxin inhibition [25].

Until now, few studies have been conducted on the repellent and insecticidal activities of bottlebrush leaf essential oils. However, there are reports on other *Callistemon* species, such as the action of weeping bottlebrush oil as an insecticidal against the aphids, *Myzus persicae* (Insecta: Hemiptera: Aphididae) [26] and the application of the powder as a fumigant against two bruchids [27]. In addition, the essential oil of *C. citrinus* was reported recently to have insecticidal and repellent properties against *T. castaneum* and *C. maculatus* [4]. Remarkably, there are no reports on the insecticidal activities of *C. lanceolatus* essential oils. Therefore, more research is required to advance our understanding of the efficient application of *C. lanceolatus* volatile oils as grain protectants for controlling storage pests.

In this regard, essential oil derived from the bottlebrush plant, *Callistemon lanceolatus*, can be effectively used as a botanical insecticide as an alternative to chemical insecticides for decreased food grain loss, thereby ensuring food's safety from storage pests. This research article aims to evaluate the chemical characterization, insecticidal, repellent activities, and phytotoxicity of *C. lanceolatus* leaf oil against the major storage pests *C. maculatus*, *L. serricorne*, and *T. castaneum* for the first time.

2. Materials and Methods

2.1. Collection of Plant Material and Extraction of Leaf Oil

The leaves of the bottlebrush *C. lanceolatus* were collected from Kerala Agricultural University, Thrissur, India (10.5449° N, 76.2864° E) in the month of August 2023. The leaf oil was extracted from 100 g dried leaves of bottlebrush by hydro-distillation in a modified Clevenger apparatus at 100 °C for 4–5 h. Anhydrous sodium sulfate was added to the natural oil to remove the water content, and it was then stored at 4 °C for later use [28]. Using the formula as follows, the yield of bottlebrush leaf oil can be determined:

$$\text{Yield of volatile oil } (\%\ v/w) = \text{VEO}/\text{WD} \times 100$$

where VEO is the volume of dried essential oil, and WD is the weight of dried leaves.

2.2. GC-MS Characterization

Chemical analysis of bottlebrush *C. lanceolatus* oils was carried out through gas chromatography–mass spectrometry (GC-MS) [23]. A Thermo Scientific TSQ 8000 Evo instrument (Thermo Scientific, Waltham, MA, USA) was used in with a capillary column and autosampler. Helium was used as the carrier gas at a flow rate of 1 mL/min with a split ratio of 1:200. TG-5MS—30 mm × 0.25 mm × 0.25 mm was employed, and the temperature program was as follows; from 12 °C/min to 125 °C for 5 min, then at 6 °C/min to 280 °C. The oven was initially set at 50 °C for one minute. Xcalibur 1.1 software was utilized for the evaluation of mass spectra data, and the components of oil were identified by comparing the mass spectra using the NIST library. The percentage peak area was used to quantify volatile components.

2.3. Test Insects

Red flour beetles were obtained from cultures stored in the laboratory. They grow well in wheat flour (100 g) and brewer's yeast (2.5% *w*/*w*). Beetles that were 30 cm × 15 cm in size were then placed inside plastic bottles. The adults were carried to new bottles following their five-day oviposition period. The adults underwent routine inspections to ensure that they were uniform. The containers were maintained at 85 ± 2% relative humidity and 30 ± 2 °C temperature for multiple repetitions. *L. serricorne*, commonly known as the cigarette beetle, was maintained in wheat flour. In plastic jars filled with wheat flour, they were raised at a relative humidity of 70 ± 3%. Adult beetles were collected from bottles and used for lab experiments [29].

C. maculatus (cowpea seed beetle), was collected from a sample of green gram. *Vigna radiata* (Fabaceae) seeds were placed in glass jars (1 L). The jars were stored at a temperature and relative humidity of 30 ± 5 °C and 75 ± 2%, respectively.

2.4. Contact Toxicity

The bottlebrush essential oil's contact toxicity was assessed using the residue film technique, with a few changes [30]. Petri plates were taken to conduct contact toxicity tests against three insects. Various concentrations of essential oil were prepared using acetone as a solvent. The concentrations were as follows: 6 mg/cm^2 to 14 mg/cm^2 for *T. castaneum*, 2 mg/cm^2 to 10 mg/cm^2 for *L. serricorne*, and 2 mg/cm^2 to 7 mg/cm^2 for *C. maculatus*. After applying 1 mL of each concentration to Petri plates using micropipettes, the plates were gently whirled to ensure even distribution. After drying, ten adults were placed in each Petri plate to observe insecticidal activity after 24 and 48 h. Three replications of each concentration were kept with control plates treated with acetone alone. Controlled mortality was accounted for by Abbott's formula, and LC_{50} and LC_{90} values were calculated.

2.5. Fumigation Toxicity

The fumigant toxicity of bottlebrush essential oil was assessed with slight modifications [28]. Fumigant chambers were air-tight glass flasks (400 mL) in which Whatman No.1 filter papers of 2 cm diameter were attached on the top. The essential oil of different concentrations (17–25 µL/L air for the red flour beetle, 1–2.5 µL/L air for the cowpea seed beetle, and 1–9 µL/L air for the cigarette beetle) was applied to filter paper using a micropipette. Then, 10 adult insects were released in each jar, ensuring no contact between the test insect and the filter paper disc. There were three replicates for each concentration along with a control. The mortality of test insects was checked after one or two days [29]. Controlled mortality was accounted for by Abbott's formula, and LC_{50} and LC_{90} values were calculated.

2.6. Repellence Activity Assay

The repellence activity of bottlebrush oil was evaluated through the area preference method [29]. In this method, Whatman No.1 filter paper discs (9 cm diameter) were placed at the bottom of the Petri plate after dividing into two halves. One half of the filter paper was treated with 500 µL of different concentrations (ranging from 1–5 mg/cm^2 both for the *T. castaneum* and *L. serricorne* and 0.1–0.5 mg/cm^2 for *C. maculatus*) and the other half with acetone as a control. Ten adult insects were taken in each Petri dish. Respectively, three replications of each concentration were assessed, and the same environmental conditions as the insect rearing (85 ± 2% relative humidity and 30 ± 2 °C temperature) were maintained. To avoid fumigation effects, the Petri dishes were closed with perforated plastic lids. The repellence percentage was found by counting the number of insects on each half of the disc every hour.

The formula that follows was used to determine the percentage of repellence (PR); [31]. PR is calculated using the formula $(NC - NT)/(NC + NT) \times 100$. NC stands for the test insects present in the control half disc and NT for the test insects present in the treatment half disc region. The results were grouped into classes ranging from class 0 (0–0.1 percent repellence) to class V (80–100 percent repellence) to estimate the repellent toxicity of bottlebrush oil based on the calculated percent repellence.

2.7. Phytotoxicity Study on Paddy Grains

A phytotoxicity study of *C. lanceolatus* oil on paddy seed germination was conducted. Briefly, 0.01% Tween-80 in distilled water was dissolved to prepare various concentrations of 500, 750, and 1000 µg/mL. Afterward, we soaked 50 g of paddy seeds in each solution for an hour. Then, 10 paddy seeds were added to filter paper and kept in sterile Petri plates. Distilled water was used for the control group. The experiment was conducted three times and at intervals of 48, 72, 96, and 120 h. Thereafter, we measured radicle length, plumule length, and the germination percentage of paddy grains and seedlings [30].

2.8. Data Analysis

LC_{50} and LC_{90} were calculated using Polo Plus software 2.0. The repellence and phytotoxicity effects were assessed using a one-way ANOVA followed by Tukey's HSD test.

3. Results

3.1. GC-MS Chemical Characterization and Yield

Hydro-distillation of dried leaves from *C. lanceolatus* yielded oil (1.02% ± 0.01, *v*/*w* of oil). The major constituents in the essential oil of *C. lanceolatus* leaves were estimated by gas chromatography–mass spectrometry (Figure 1). Briefly, 1,8-cineole (19.17%), α-terpineol (11.51%), α-pinene (10.28%), and α-Phellandrene (9.55%) were the principal components in the essential oil. The complete composition contained a total of 25 compounds, which are represented in Table 1.

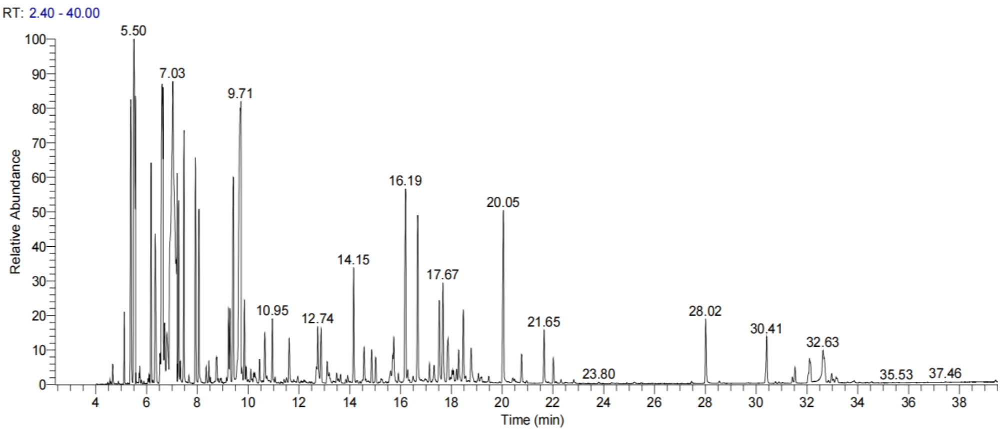

Figure 1. GC-MS chromatograms of *Callistemon lanceolatus* leaf oil.

Table 1. Chemical constituents of *Callistemon lanceolatus* leaf oil.

Peak No.	[a] Retention Time	Compounds	%Area	[b] RSI	Identification Method
1.	5.50	α-pinene	10.28	803	MS
2.	6.17	3-carene	2.78	888	MS
3.	6.34	α-Myrcene	2.73	893	MS
4.	6.61	α-Phellandrene	9.55	920	MS
5.	7.03	1,8-cineole	19.17	902	MS
6.	7.19	Eucalyptol	1.14	905	MS
7.	7.26	α-ocimene	1.90	928	MS
8.	7.47	ς-terpinene	3.44	912	MS
9.	7.92	2-Carene	2.82	915	MS
10.	8.06	1,6-Octadien-3-ol, 3,7-dimethyl-	2.48	944	MS
11.	9.23	Borneol	1.08	942	MS
12.	9.41	Terpinen-4-ol	3.89	926	MS
13.	9.70	α-terpineol	11.51	912	MS
14.	12.74	Eugenol	1.26	956	MS
15.	14.15	Caryophyllene	1.83	958	MS
16.	15.73	α-Guanine	1.29	912	MS
17.	16.19	Dur hydroquinone	3.64	917	MS
18.	16.67	Ethanone, 1-(2,4,5-tri methoxyphenyl)-	3.05	904	MS
19.	17.52	Spathulenol	1.52	955	MS
20.	17.67	Globulol	2.03	964	MS
21.	18.47	2-Naphthalenemethanol	1.31	943	MS
22.	20.04	4,6-di-t-Butylpytagallol	3.42	909	MS
23.	28.02	Phytol	1.20	902	MS
24.	32.63	ς-Sitosterol	1.50	961	MS

[a] Retention time. [b] RSI: Reverse similarity index

3.2. Contact Toxicity

Contact toxicity bioassays revealed that bottlebrush leaf oils at varying concentrations significantly affected *T. castaneum*, *L. serricorne*, and *C. maculatus* adults. Compared to red flour beetles, *L. serricorne,* and *C. maculatus* were more sensitive to *C. lanceolatus* leaf oil at 24 and 48 h after exposure, even at low concentrations. Probit analysis revealed that the LC_{50} and LC_{90} values for *C. lanceolatus* oils against the red flour beetle adults were 1.35 mg/cm^2 and 2.45 mg/cm^2, respectively, following 24 h of exposure. Similarly, after 48 h of exposure to red flour beetles, *C. lanceolatus* oil showed LC_{50} and LC_{90} values of 0.97 and 1.51 mg/cm^2, respectively. The LC_{50} and LC_{90} readings for adult *L. serricorne* were 0.52 mg/cm^2 and 1.35 mg/cm^2 each, after 24 h. The LC_{50} and LC_{90} values after two days of contact with *L. serricorne* were 0.50 mg/cm^2 and 1.08 mg/cm^2, respectively. For *C. maculatus* adults, the LC_{50} values and LC_{90} values after 24 h of exposure were 0.580 mg/cm^2 and 1.125 mg/cm^2, respectively. Likewise, *C. lanceolatus* oil exhibited LC_{50} and LC_{90} readings of 0.315 and 0.845 mg/cm^2, respectively following 48 h of exposure to *C. maculatus*. The current research suggests that adult *T. castaneum*, *C. maculatus,* and *L. serricorne* were significantly affected by the contact toxicity of essential oils derived from *C. lanceolatus* leaves (Table 2).

Table 2. Lethal doses of *Callistemon lanceolatus* leaf oils on contact toxicity against major storage insect pests at various exposure intervals.

Test Storage Insects	Exposure Time (h)	LC_{50} (mg/cm^2)	LC_{90} (mg/cm^2)	Slope ± SEM [a]	χ^2 (df)
T. castaneum	24	1.35 (1.15–2.04)	2.45 (1.87–5.14)	1.16 ± 0.38	0.75 (3)
	48	0.97 (0.79–1.11)	1.51 (1.28–2.68)	2.38 ± 0.84	0.01 (3)
L. serricorne	24	0.52 (0.35–0.66)	1.35 (1.08–2.10)	1.55 ± 0.38	0.03 (3)
	48	0.50 (0.30–0.64)	1.08 (0.86–2.19)	2.40 ± 0.84	0.04 (3)
C. maculatus	24	0.58 (0.45–1.15)	1.12 (0.79–2.86)	2.35 ± 0.82	0.66 (3)
	48	0.31 (0.21–0.43)	0.84 (0.62–1.69)	2.41 ± 0.75	0.10 (3)

[a] SEM: Mean standard error. χ^2: chi-square.

3.3. Fumigation Toxicity

The cigarette beetle, red flour beetle, and cowpea seed beetle exhibit intense fumigation toxicity to the volatile oils of dried bottlebrush leaves. *C. maculatus* had higher mortality at a very low concentration (1–2.5 µL/L air) across different time intervals compared to the cigarette beetle and red flour beetle. The mortality rate of both test store grain insects was significantly affected by the combination of *C. lanceolatus* oil concentration and exposure period. According to Probit analysis, the LC_{50} and LC_{90} values of *T. castaneum* after 24 h were 22.601 and 32.349 µL/L air, respectively. Similarly, the lethal concentrations of *T. castaneum* after 48 h of exposure yielded LC_{50} and LC_{90} values of 19.824 and 31.422 µL/L air, respectively. The LC_{50} value of bottlebrush leaf oil for *C. maculatus* and *L. serricorne* was 1.43 and 5.48 µL/L air, respectively, after 24 h, indicating the highest fumigation toxicity. After 48 h of fumigant exposure, the LC_{50} value for the test insects, *C. maculatus* and *L. serricorne* was 1.27 and 4.17 µL/L air, respectively. Fumigation is directly correlated with concentration and time exposed (Table 3).

Table 3. Lethal doses of *Callistemon lanceolatus* leaf oils on fumigation toxicity against major storage insect pests at various exposure intervals.

Insects	Time Interval (h)	LC_{50} (µL/L Air)	LC_{90} (µL/L Air)	Slope ± SEM [a]	χ^2 (df)
T. castaneum	24	22.60 (20.84–26.27)	32.34 (27.93–44.82)	0.13 ± 0.33	0.40 (3)
	48	19.84 (17.79–22.43)	31.42 (26.75–47.62)	0.11 ± 0.03	2.03 (3)
L. serricorne	24	5.48 (4.78–6.19)	9.23 (8.14–10.82)	0.34 ± 0.04	2.83 (3)
	48	4.17 (4.07–5.76)	8.52 (7.24 -10.75)	0.27 ± 0.04	2.61 (3)
C. maculatus	24	1.43 (1.22–1.61)	2.24 (2.08–2.77)	1.43 ± 0.25	0.02 (2)
	48	1.27 (1.02–1.41)	2.09 (1.94–2.95)	1.04 ± 0.25	0.20 (2)

[a] SEM: Mean standard error. χ^2: chi-square.

3.4. Repellent Activity

The repellent activity of *C. lanceolatus* leaf oils against the *T. castaneum*, *L. serricorne*, and *C. maculatus* at various exposure times is provided in the table below (Table 4). *Tribolium castaneum* adults exhibited better repellent activity at different concentrations and exposure times. In the case of *T. castaneum*, the PR values were more than 50% from 1 to 6 h post-exposure when the concentrations were 4 and 5 mg/cm^2 (Figure 2). When the *T. castaneum* was exposed to a dose of 5 mg/cm^2, the mean repellence was 81 (Class V). Similarly, at a concentration of 5 mg/cm^2, adult *L. serricorne* showed a mean repellence of 66.6% (Class IV) 1–6 h after exposure (Figure 3). A mean percent repellence of 60.5 was found for *C. lanceolatus* volatile oil against *C. maculatus* at a dose of 0.5 mg/cm^2, 1 to 6 h after exposure (Class IV) (Figure 4). In the research study, *T. castaneum* exhibited intense repellent activity (Class II-V) every hour. *C. lanceolatus* oil showed good repellence (Class II-IV) when tested against *C. maculatus* and *L. serricorne*. The results indicate that the exhibits repellent potential of essential oil against stored product pests.

Table 4. Mean repellent percentage of *Callistemon lanceolatus* oil against red flour beetle, cigarette beetle and cowpea seed beetle.

Test Insects	Dose (mg/cm^2)	Mean Repellence	Repellent Class
T. castaneum	1	36.63 ± 6.99 [d]	II
	2	48.28 ± 5.85 [c]	III
	3	60.51 ± 10.21 [b]	IV
	4	70.53 ± 12.88 [b]	IV
	5	81.08 ± 4.99 [a]	V
L. serricorne	1	24.96 ± 13.95 [c]	II
	2	42.18 ± 13.60 [b]	III
	3	48.28 ± 5.85 [b]	III
	4	54.96 ± 14.56 [ab]	III
	5	66.63 ± 10.51 [a]	IV
C. maculatus	0.1	28.30 ± 10.27 [b]	II
	0.2	36.06 ± 7.42 [b]	II
	0.3	53.30 ± 6.34 [a]	III
	0.4	59.41 ± 9.03 [a]	III
	0.5	60.51 ± 10.21 [a]	IV

[a, b, c, d] Means in the same column that are preceded by the same letter do not differ significantly ($p < 0.05$).

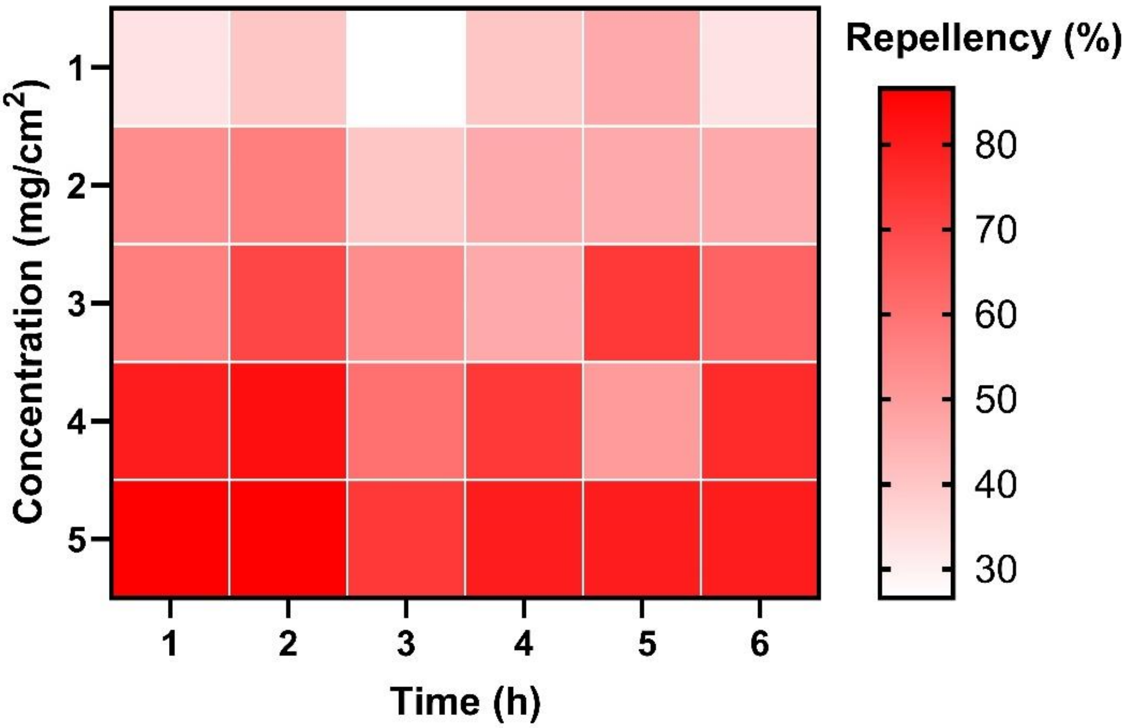

Figure 2. Mean repellent percentage of *Callistemon lanceolatus* leaf oil to control *Tribolium castaneum*.

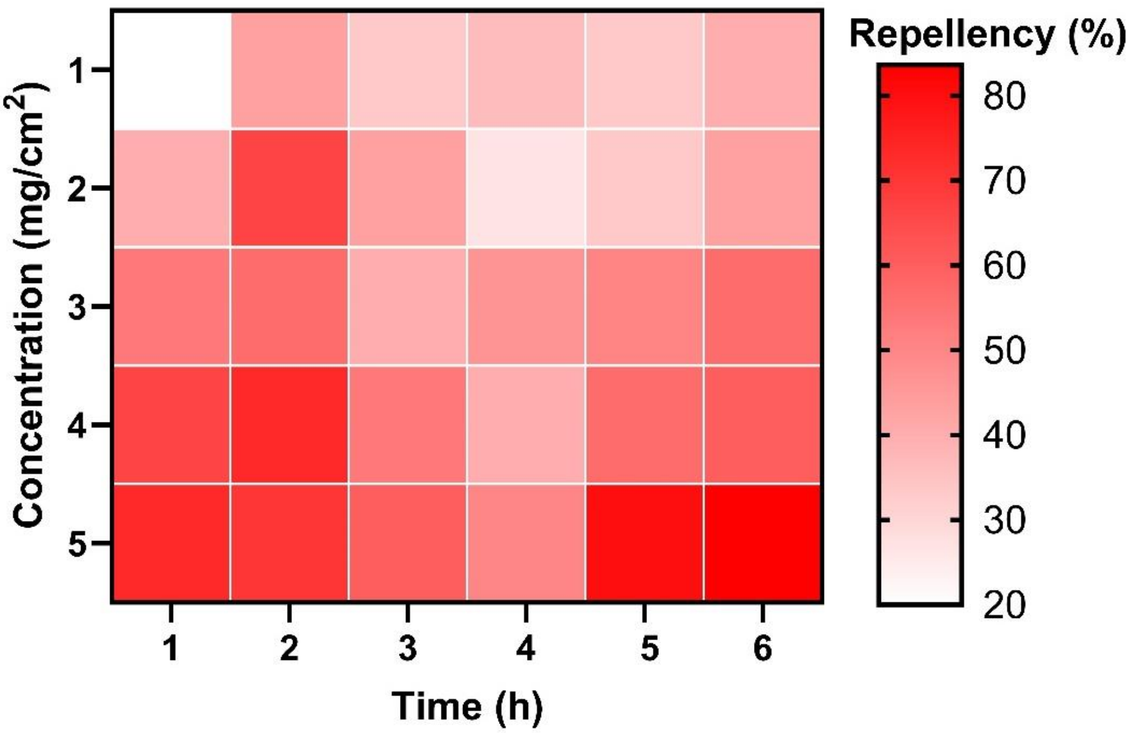

Figure 3. Mean repellent percentage of *Callistemon lanceolatus* leaf oil to control *Lasioderma serricorne*.

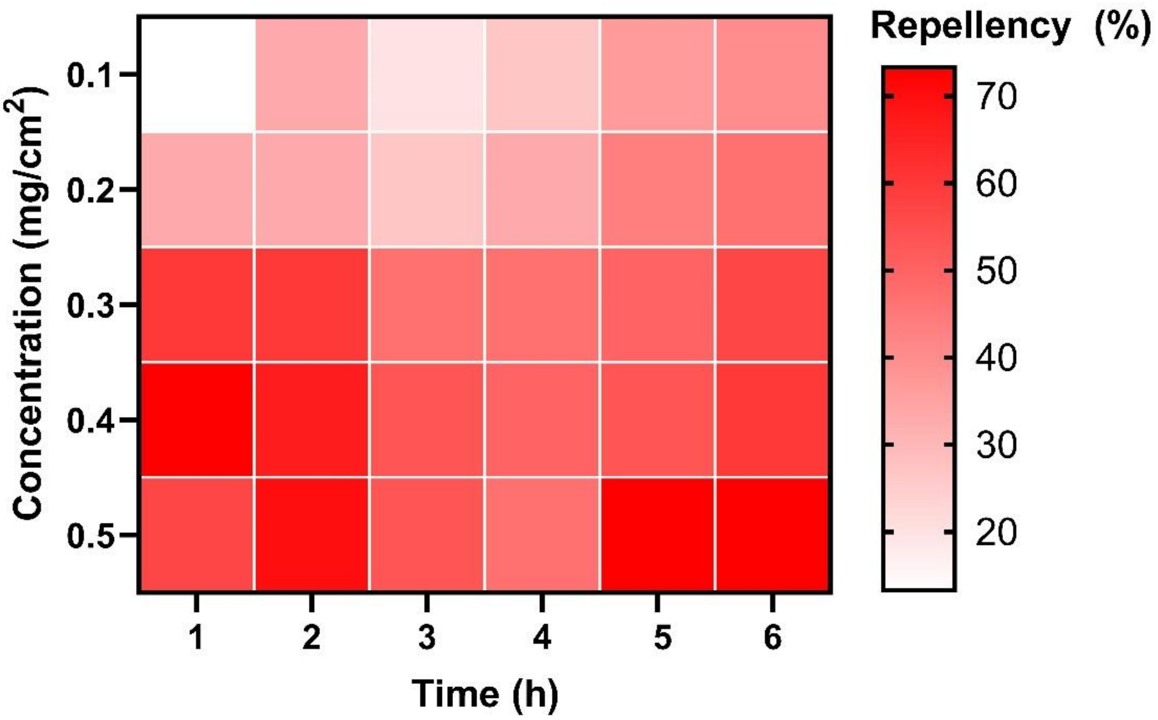

Figure 4. Mean repellent percentage of *Callistemon lanceolatus* leaf oil to control *Callasobruchus maculatus*.

3.5. Phytotoxicity of Callistemon lanceolatus Leaf Oils

The effects of *C. lanceolatus* leaf essential oil at various doses (500, 750, and 1000 µg/mL) on paddy seed germination at various times (48 h, 72 h, 96 h, and 120 h) are illustrated below in Table 5. The data shows that the germination of paddy seeds treated with *C. lanceolatus* leaf oil is comparable to the control and positive control treatments. The percentage of treated seeds that germinate compared to control seeds is not statistically different ($p > 0.05$). According to the study's findings, applying *C. lanceolatus* leaf essential oil at the measured quantities had no negative effects on rice seed.

Table 5. Phytotoxicity effects of *Callistemon lanceolatus* leaf oil on germination percentage of paddy seeds.

Concentration (µg/mL)	Seed Germination Percentage after Treatment			
	48 h	**72 h**	**96 h**	**120 h**
500	73.3 ± 25.1 [b]	80.0 ± 10 [a]	86.6 ± 5.7 [a]	96.6 ± 5.7 [a]
750	76.6 ± 25.1 [b]	96.5 ± 5.7 [a]	96.6 ± 5.7 [a]	96.6 ± 5.7 [a]
1000	70.0 ± 20.0 [b]	93.3 ± 5.7 [a]	93.3 ± 5.7 [a]	96.6 ± 5.7 [a]
[c] Control	86.6 ± 5.7 [a]	93.3 ± 5.7 [a]	93.3 ± 5.7 [a]	100 ± 0 [a]

[c] Distilled water; [a,b] Means in the same column that is preceded by the same letter do not differ significantly ($p < 0.05$).

Various concentrations of *C. lanceolatus* leaf essential oil (500, 750, and 1000 µg/mL) were examined for 120 h to observe how they affected the growth of paddy seedlings. The radicle length and plumule length were measured at 48, 72, 96, and 120 h after treatment (Tables 6 and 7). The radicle length, as well as the plumule length of paddy seeds, were unaffected by essential oil when compared to the control. There were no significant differences among the treatments and control.

Table 6. Phytotoxicity effects of *Callistemon lanceolatus* leaf oil on seedling radicle growth of treated paddy grains.

Concentration (µg/mL)	Seedling Growth of Treatments after			
	48 h	72 h	96 h	120 h
	Radicle (mm)			
500	0.9 ± 0.57 [a]	9.7 ± 4.7 [a]	13.7 ± 5.0 [a]	30 ± 5.0 [a]
750	1.0 ± 0.0 [a]	9.1 ± 2.98 [a]	14.1 ± 1.1 [a]	31 ± 2.6 [a]
1000	1.0 ± 0.09 [a]	10.8 ± 4.51 [a]	13.1 ± 4.4 [a]	30.5 ± 5.6 [a]
[b] Control	1.2 ± 0.57 [a]	11.4 ± 1.05 [a]	14.8 ± 0.5 [a]	31.1 ± 8.4 [a]

[b] Distilled water; [a] Means in the same column that are preceded by the same letter do not differ significantly ($p < 0.05$).

Table 7. Phytotoxicity effects of *Callistemon lanceolatus* leaf oil on seedling plumule growth of treated paddy grains.

Concentration (µg/mL)	Seedling Growth of Treatments after			
	48 h	72 h	96 h	120 h
	Plumule (mm)			
500	0.0 ± 0.0 [a]	0.5 ± 0.0 [a]	7.3 ± 0.83 [a]	10.3 ± 0.85 [a]
750	0.0 ± 0.0 [a]	0.9 ± 0.0 [a]	8.7 ± 1.02 [a]	9.13 ± 0.5 [a]
1000	0.0 ± 0.0 [a]	0.6 ± 1.03 [a]	7.3 ± 0.65 [a]	9.63 ± 0.65 [a]
[b] Control	0.0 ± 0.0 [a]	1.0 ± 1.00 [a]	8.4 ± 0.60 [a]	10.2 ± 1.2 [a]

[b] Distilled water; [a] Means in an identical column that is preceded by the same letter do not differ substantially ($p < 0.05$).

4. Discussion

This is the pioneering research to study the efficacy of repellent and insecticidal activities associated with the leaves of *C. lanceolatus* oil. The yield of oil is 1.02% ± 0.01(v/w) was rather high in the experiment. The raw materials are available in plenty as the plant grows luxuriantly in gardens in India [25]. Our study resulted in higher essential oil yield from *C. lanceolatus* leaves than the yields of oil extracted from the leaves of *C. rigidus*, *C. citrinus*, and *C. viminalis* (0.43%, 0.84%, and 0.41% (v/w)), respectively [25]. The *C. lanceolatus* oil can be extracted in large quantities since the raw materials are readily available and yield a significant amount of essential oils (7.6 mL kg^{-1}). Therefore, it would be cost-effective to use essential oils for insecticidal activities in storage [32]. The high essential oil yield from our study suggests that the volatile oils extracted from *C. lanceolatus* leaves can be considered for biopesticide formulation.

According to the GC-MS analysis, the main components identified in the essential oil from bottlebrush leaves were 1,8-cineole (19.17%), α-pinene (10.28%), α-terpineol (11.51%), α-Phellandrene (9.55%). Until now, there have been only a few reports regarding the chemical constitution of oil from *C. lanceolatus* leaves [19,21,33,34]; 1,8-cineole and α-pinene were the most abundant compounds, followed by α-Phellandrene, limonene, and α-terpineol, which are in line with our investigation. Moreover, 1,8-cineole (52.1%), α-Terpineol (14.7%), and eugenol (14.2%) were important compounds present in the essential oil of *C. citrinus* from Nepal, which were similar to those in India [35]. It is important to emphasize that the chemical composition of the oil varies qualitatively and quantitatively with the growth stage of the plant, environment factors, genetics, soil type, climate, geographic location, cultivars, and time of harvest [31,33,36]. The difference in chemical composition is the main reason for the biological activities of constituents present in essential oils [37]. The chemical constituents and their site-specific actions are the reason for using the essential oil against stored product pests when applied as fumigant, contact, and repellent [38].

The repellent activity, contact toxicity assay, and fumigant activity exert discernible influences on the essential oil concentration, exposure duration, and target species [7]. Until now, very few studies have reported on the fumigation, repellent, contact toxicity, and insecticidal effects of *C. lanceolatus* oil on insects in stored products. However, there have been studies conducted on the fumigation, repellence, ovicidal, antifeedants, and larvicidal effects of *C. lanceolatus* leaf oil on *C. chinensis* in stored products [32]. Additionally, previous studies have shown the insecticidal activity of several other *Callistemon* species, such as *C. vimanalis* oil against the aphid, *Myzus persicae* (Insecta: Hemiptera: Aphididae) [26], which was utilized as a powder and fumigant to control two bruchids [27]. In addition to this, the repellence and insecticidal properties of the leaf oil of the *C. citrinus* against red flour beetle (*T. castaneum*) and cowpea seed beetle (*C. maculatus*) [4] were reported. Different doses of *C. lanceolatus* extract were used to assess fumigancy, contact toxicity, and insect mortality [39]. According to earlier results, crude *C. lanceolatus* extracts and isolated compounds exhibited notable antibacterial, antioxidant, anti-diabetic, and insecticidal effects [21].

The compound 1,8-cineole is rich in *C. lanceolatus* essential oils and is well-known for having strong insecticidal properties. It is extremely poisonous to beetle species that attack stored goods; it is repellent to them [7]. Furthermore, 1,8-cineole inhibits the synthesis of juvenile hormones when it comes into contact with insects. It prevents acetylcholinesterase from functioning, by occupying the hydrophobic position in the active center of the enzyme [40]. In addition, 1,8-cineole has demonstrated 100% insect mortality, even at a lower dose of 0.1 μL/mL. This oil functions as a potential fumigant agent both in antifeedant activity and as an oviposition deterrent [32]. Another study has proven that *C. lanceolatus* leaf extract minimized the growth and ceased the emergence of fall armyworm adults [21]. Additionally, α-pinene, the other main compound of *C. lanceolatus* essential oil, has biological activity against a variety of insect species [21]. Similarly, insecticidal properties were also noted with α-terpineol in *C. viminalis* essential oil [41]. Chemical compounds with lower molecular weight volatilize more rapidly and accumulate in confined spaces [42]. Because of this, insects inhale more of these compounds, which results in a higher mortality rate [43].

This study demonstrates how well the oil from *C. lanceolatus* leaves could elicit contact toxicity in adult *C. maculatus*, *L. serricorne*, and *T. castaneum*. To the best of our knowledge, there have been no studies conducted on the contact toxicity activities brought on by oils extracted from *C. lanceolatus* leaves against stored grain pests. Based on our research, the toxicity of the *C. lanceolatus* volatile oil was found to be dose-dependent, becoming more pronounced in cigarette beetle (*L. serricorne)* and cowpea seed beetle (*C. maculatus)* adults with higher dosages of the essential oil. In our research, *C. maculatus* exhibited an LC_{50} value of 0.315 mg/cm^2 after 48 h. The contact toxicity of leaves of another species of *Callistemon*, that is, *C. viminalis,* against *C. maculatus* (Coleoptera: Bruchidae) adults was evaluated (LC_{50} value of 0.170 mg/cm^2), and the results support our findings [27], *C. rigidus* essential oil is toxic to cowpea beetles *(C. maculatus*) [44], and *C. citrinus* oil at 100% concentration showed 100% mortality within an hour when applied against *L. serricorne* [6] and to red flour beetle [45] in contact toxicity assays. The findings of other studies support the high contact toxicity of *C. viminalis* to *S. oryzae,* with LC_{50} values of 0.09 mg/cm^2 [43]. Previous research on the effectiveness of *Cyperus rotundus* oil in a contact assay against *Trogloderma granarium*, *Oryzaephilus surinamensis*, and *C. maculatus* (LC_{50} value = 0.36, 0.51, and 0.2 mg/cm^2) is comparable to and concordant with our results [46]. The research's findings suggest that the bioactive components in the evaluated essential oils may interact with a variety of insect target sites and turn them more toxic [47].

According to the fumigant toxicity testing results, *C. maculatus* and *L. serricorne* are more toxic to fumigation exposure of *C. lanceolatus* essential oils, and the mortality rate was also high at a modest concentration. The unique characteristics of these essential oils, with fumigant toxicity, repellent activity, and oviposition deterrent activity, make *C. lanceolatus* available to use as a potential fumigant to control the pests of grains/pulses [33]. Our result indicates the intense fumigant activity of *C. lanceolatus* essential oil when applied against *C. maculatus* at a smaller dose (1–2.5 μL/L air), with LC_{50} values of 1.43 μL/L air and 2.32 μL/L air at 24 and 48 h. Similarly, a study conducted on fumigation toxicity of oil from *C. citrinus* against *C. maculatus* also exhibited intense fumigant toxicity, where the LC_{50} values of the essential oil were 12.88 μL.L^{-1} for males and 84.4 μL.L^{-1} for females. The results differed with the duration of the insect's exposure to the oil [16]. The crimson bottlebrush has a natural oil composition and is effective at deterring red flour beetles [4]. The lethal concentration (LC_{50}) of the bottlebrush oil was 37.05 µL/L and showed 100% mortality in adult red flour beetles at 9 h after treatment at a concentration of 160 µL/L. The essential oil from the leaves of *C. vimanalis* can be used as a fumigant agent against *A. obtectus* and *C. maculatus*, where the LC_{50} values calculated were 0.019 and 0.011 µL/cm^3 towards *A. obtectus* and *C. maculatus* after 12 h exposure [27].

The *C. vimanalis* oil was more toxic to the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae), through fumigation, wherein the LC_{50} values were 24.60 µL/L air. It was reported that the oil of *C. viminalis* (LC_{50} = 50 µL/L air) had a high mortality rate against *T. confusum* after fumigation [48]. The LC_{50} values for Italian cypress, *Cupressus sempervirens* (Pinales: Cupressaceae); weeping bottlebrush, *C. vimanalis* (Myrtales: Myrtaceae); lemon, Citrus lemon (Sapindales: Rutaceae); sweet orange, *Citrus sinensis* (Sapindales: Rutaceae); and wild marjoram, *Origanum vulgare* (Lamiales: Lamiaceae) were 17.16, 16.17, 9.89, 19.65, and 1.64 µL/L air, respectively, showing a potent toxic effect on rice weevil upon a fumigation assay [43]. Some 6 of the 42 essential oils isolated from Myrtaceae species found in Australia were found to have higher fumigation toxicity against *T. castaneum*; rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae); and a lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae), which is in support of our findings. These were the nicholii, codonocarpa, blakelyi, sieberi, fulgens, and armillaris essential oils from the *Eucalyptus* species. The LC_{50} and LC_{95} values against adults of *S. oryzae* for the chosen essential oils were 19.0 and 30.6 and 43.6 and 56.0 µL/L air, respectively [49]. Numerous studies using oils isolated from various plants or plant components have also demonstrated effective fumigation effects against storage pests even at lower concentrations [50–52]. Recently, bio-fumigation has gained more acceptance when compared to synthetic chemical insecticides because of its fewer toxic effects and better control of stored product insects [4,28]. Fumigation is one of the fastest, most efficient, and practical methods available for preventing pest infestation in feedstocks, stored food grains, and other agricultural products [4].

Bottlebrush leaves were tested for their repellent qualities against these stored grain pests, and the repellence index (%RI) was observed at 24 h. Likewise, bottlebrush leaf oil was highly effective in repelling red flour beetles (*T. castaneum*) and cigarette beetles (*L. serricorne*) (PR > 50%) at a dose of 5 mg/cm^2. This was similar to previous studies in which *L. serricorne* was found to be highly susceptible to the repellent and insecticidal effects of bottlebrush essential oil [6]. Additionally, our study showed a strong repellence when *C. lanceolatus* was applied to *C. maculatus* at a dose of 0.5 mg/cm^2 in our study. This result is consistent with repellent properties that have been reported with the essential oil of *C. lanceolatus* against *C. chinensis* at a dose of 150 µL [32]. Similar repellent properties have also been studied for essential oils from leaves of other species; for instance, *C. citrinus* exhibited a good repellence against *C. maculatus*, and this was observed to be dose-dependent ($p < 0.001$) [16]. *Tribolium castaneum* had a maximum repellence of 93.3% at a concentration of 20 µL after 24 h of observation [4], and *C. viminalis* repelled *Rhipicephalus* spp. [53].

Essential oils from various plants, including iruveriya, *Plectranthus zeylanicus* (Larniales: Lamiaceae), dalchini, *Cinnamomum zeylanicum* (Laurales: Lauraceae), lime berry, *Micromelum minutum* (Sapindales: Ru-taceae), and jamboa, *Citrus maxima* (Sapindales: Rutaceae), have also been shown to have similar insect-repellent properties against the red flour beetle (*T. castaneum*) and pulse beetle (*C. chinensis*) [8,28,54]. Further, it has been proved that *C. rotundus* essential oil strongly repels *C. maculatus*, *O. surinamensis*, and *T. granarium* [46]. In all these studies, the repellence elevated with the increase in the dose of the oil for longer exposure times [7]. Previous research works have examined the ability of several volatile oils to repel various stored grain pests. The concentration used and the exposure time had a major impact on the repellence effect of aromatic oils, as was seen in our study [7].

In the current investigation, paddy seeds were exposed to the phytotoxicity effects of *C. lanceolatus* essential oil. The results showed that the essential oil was safe for use in agriculture because it did not hinder rice seed germination at any of the concentrations or periods. These results are in line with those of earlier research that demonstrated the safety of many citrus species' essential oils from the Rutaceae family on the germination of other plants, including wheat, *Triticum aestivum* (Poales: Poaceae), and paddy, *Oryza sativa* (Poales: Poaceae) [28,55]. Furthermore, the leaf oil had no significant effect on the radicle length of rice seeds that had been treated, showing that it has no phytotoxic effect on paddy seedlings. These observations are consistent with previous studies on the phytotoxic effects of *C. viminalis* leaf oil on seedling growth and seed germination [29]. However, there are some study reports on the phytotoxic effects of essential oils [56]. There, phytotoxicity mainly occurred due to the presence of monoterpenes, especially cineole. Similarly, when lemon-scented gum (*Eucalyptus citriodora* (Myrtales: Myrtaceae)) leaf oil was applied to wheat, seed germination decreased significantly [56].

As a result, our findings show that the oil from the leaves of *C. lanceolatus* has the potential to replace chemical pesticides in the fight against the red flour beetle (*T. castaneum)*, cigarette beetle (*L. serricorne*), and cowpea seed beetle (*C. maculatus)* in stored grain products, largely because of its high fumigation, contact toxicity, and repelling properties. It ensures a natural supply of botanical pesticides [16]. Recently, natural insecticides have become more popular because they have fewer negative effects, and more attention is being paid to the creation of organic pesticides to prevent food loss from stored grain pests.

5. Conclusions

Essential oils are promising natural chemicals that can be used to control stored grain pests. According to our research, *C. lanceolatus* leaf oil exhibited remarkable insecticidal properties against the main stored product insects, highlighting the essential oil's potential application (without any phytotoxicity effects) to stored grain systems and units. The *C. lanceolatus* leaf oil exhibited notable repellent and insecticidal activities, demonstrating potent effects in repellence, contact toxicity, and fumigation toxicity against the tested insect species, namely the red flour beetle (*T. castaneum*), cigarette beetle (*L. serricorne*), and cowpea seed beetle (*C. maculatus*). The essential oil-containing phytochemicals are possible alternative chemical insecticides and can be used as botanical pesticides. This research aims to ensure food safety by effectively managing storage pests through natural insecticides (used as an alternative to chemical insecticides). Additionally, there is a need for studies on the wide application of *C. lanceolatus* essential oil to control more serious and commercial pests in stores. Furthermore, research studies are required to elevate food quality and safety with the use of essential oils loaded with nanoparticles.

Author Contributions: Conceptualization, B.P. and N.U.V.; methodology, B.P., N.U.V. and T.A.A.; software, N.M.; validation, B.P. and N.U.V.; formal analysis, N.U.V. and T.A.A.; investigation, T.A.A.; writing—original draft preparation, T.A.A.; writing—review and editing, B.P., N.U.V., N.M., R.S.B. and R.S.; visualization, N.U.V.; supervision, B.P., R.S., R.S.B. and N.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ongoing research related to this work.

Acknowledgments: The authors express their gratitude to the Kerala Agricultural University, Thrissur, India; University of Tabuk, Saudi Arabia; University of Verona, Italy; and Alexandria University, Egypt, for their facilities and other research support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Imathiu, S. Benefits and food safety concerns associated with consumption of edible insects. *NFS J.* **2020**, *18*, 1–11. [CrossRef]
2. Tripathi, A.K. Pests of Stored Grains. In *Pests and Their Management*; Springer Nature Singapore Pte Ltd.: Singapore, 2018; pp. 311–359. [CrossRef]
3. Upadhyay, R.; Ahmad, S. Management Strategies for Control of Stored Grain Insect Pests in Farmer Stores and Public Ware Houses. *World J. Agric. Sci.* **2011**, *7*, 527–549.
4. Ramachandran, M.; Baskar, K.; Jayakumar, M. Essential Oil Composition of *Callistemon citrinus* (Curtis) and Its Protective Efficacy against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *PLoS ONE* **2022**, *17*, e0270084. [CrossRef]
5. Campbell, J.F.; Athanassiou, C.G.; Hagstrum, D.W.; Zhu, K.Y. *Tribolium castaneum*: A Model Insect for Fundamental and Applied Research. *Annu. Rev. Entomol.* **2022**, *67*, 347–365. [CrossRef]
6. Viswan, K.; Rahana, V.; Pushpalatha, E. Repellent and Adulticidal Efficacy of Essential Oils of Two Indigenous Plants from Myrtaceae Family Against *Lasioderma serricorne* F. *J. Biopestic.* **2013**, *7*, 70–74.
7. Zandi-Sohani, N.; Hojjati, M.; Carbonell-Barrachina, Á.A. Insecticidal and Repellent Activities of the Essential Oil of *Callistemon citrinus* (Myrtaceae) against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Neotrop. Entomol.* **2012**, *42*, 89–94. [CrossRef] [PubMed]
8. Balachandra, B.A.H.E.; Pathirathna, P.U.; Paranagama, P.A. Control of Stored Grain Pest, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) Using the Essential Oil Isolated from *Plectranthus zeylanicus*. *Nat. Prod. Res.* **2012**, *26*, 2219–2222. [CrossRef]
9. Samada, L.H.; Tambunan, U.S.F. Bio pesticides as Promising Alternatives to Chemical Pesticides: A Review of Their Current and Future Status. *Online J. Biol. Sci* **2020**, *20*, 66–76. [CrossRef]
10. Ngegba, P.M.; Cui, G.; Khalid, M.Z.; Zhong, G. Use of Botanical Pesticides in Agriculture as an Alternative to Synthetic Pesticides. *Agriculture* **2022**, *12*, 600. [CrossRef]
11. Carrascosa, M.; Sánchez-Moreno, S.; Alonso-Prados, J.L. Effects of Organic and Conventional Pesticides on Plant Biomass, Nematode Diversity and the Structure of the Soil Food Web. *Nematology* **2015**, *17*, 11–26. [CrossRef]
12. Narayanankutty, A.; Visakh, N.U.; Sasidharan, A.; Pathrose, B.; Olatunji, O.J.; Al-Ansari, A.; Alfarhan, A.; Ramesh, V. Chemical Composition, Antioxidant, Anti-Bacterial, and Anti-Cancer Activities of Essential Oils Extracted from *Citrus limetta* Risso Peel Waste Remains after Commercial Use. *Molecules* **2022**, *27*, 8329. [CrossRef]
13. Albaqami, J.J.; Hamdi, H.; Narayanankutty, A.; Visakh, N.U.; Sasidharan, A.; Kuttithodi, A.M.; Famurewa, A.C.; Pathrose, B. Chemical Composition and Biological Activities of the Leaf Essential Oils of *Curcuma longa*, *Curcuma aromatica* and *Curcuma angustifolia*. *Antibiotics* **2022**, *11*, 1547. [CrossRef]
14. Kumar, S.; Kumar, A.; Gupta, P.; Srivastava, Y. Plants Based Insecticides as Promising Source of Bio pesticides—A Review. *ISSN* **2021**, *11*, 51–57. [CrossRef]
15. Cascaes, M.M.; Guilhon, G.M.S.P.; Andrade, E.H.d.A.; Zoghbi, M.d.G.B.; Santos, L.d.S. Constituents and Pharmacological Activities of Myrcia (Myrtaceae): A Review of an Aromatic and Medicinal Group of Plants. *Int. J. Mol. Sci.* **2015**, *16*, 23881–23904. [CrossRef] [PubMed]
16. Zandi-Sohani, N.; Hojjati, M.; Carbonell-Barrachina, A.A. Volatile Composition of the Essential Oil of *Callistemon citrinus* Leaves from Iran. *J. Essent. Oil-Bear. Plants* **2012**, *15*, 703–707. [CrossRef]
17. Chachad, D.P.; Laiane, A.; Uniyal, K.; Varma, U.; Jadhav, P.; Satvekar, T.; Ghag-Sawant, M.; Mondal, M.A.; Doshi, N. Chemical Characterization; Antimicrobial and Larvicidal Activity of Essential Oil from *Callistemon citrinus* (Bottle Brush) Leaves. *bioRxiv* **2021**. [CrossRef]
18. Ahmad, K.; Hafeez, Z.B.; Bhat, A.R.; Rizvi, M.A.; Thakur, S.C.; Azam, A.; Athar, F. Antioxidant and Apoptotic Effects of *Callistemon lanceolatus* Leaves and Their Compounds against Human Cancer Cells. *Biomed. Pharmacother.* **2018**, *106*, 1195–1209. [CrossRef] [PubMed]
19. Nazreen, S.; Mahboob Alam, M.; Hamid, H.; Ali, M.; Sarwar Alam, M. Chemical Constituents with Antimicrobial and Antioxidant Activity from the Aerial Parts of *Callistemon lanceolatus* (Sm.) Sweet. *Nat. Prod. Res.* **2019**, *34*, 3275–3279. [CrossRef]
20. Park, S.S.; Lim, W.; Jeong, W.; Bazer, F.W.; Lee, D.; Song, G. Sideroxylin (*Callistemon lanceolatus*) Suppressed Cell Proliferation and Increased Apoptosis in Ovarian Cancer Cells Accompanied by Mitochondrial Dysfunction, the Generation of Reactive Oxygen Species, and an Increase of Lipid Peroxidation. *J. Cell. Physiol.* **2018**, *233*, 8597–8604. [CrossRef]
21. Kumar, R.; Pandey, A. Antioxidant and Anticancer Activity of *Callistemon lanceolatus* Extracts. *Free Radic. Biol. Med.* **2019**, *145*, S69–S70. [CrossRef]
22. Kumar, S.; Kumar, V.; Prakash, O. Pharmacognostic Study and Anti–Inflammatory Activity of *Callistemon lanceolatus* Leaf. *J. Trop. Biomed.* **2011**, *1*, 177–181. [CrossRef]
23. Sowndhararajan, K. A Review of the Chemical Composition and Biological Activities of *Callistemon lanceolatus* (Sm.) Sweet. *J. Appl. Pharm. Sci.* **2021**, *11*, 65–73. [CrossRef]
24. Kavitha, K.S.; Satish, S. Antibacterial activity of *Callistemon lanceolatus* DC. against human phytopathogenic bacteria. *J. Pharm. Res.* **2013**, *7*, 235–240. [CrossRef]
25. Shukla, R.; Singh, P.; Prakash, B.; Dubey, N.K. Antifungal, Aflatoxin Inhibition and Antioxidant Activity of *Callistemon lanceolatus* (Sm.) Sweet Essential Oil and Its Major Component 1,8-Cineole against Fungal Isolates from Chickpea Seeds. *Food Control* **2012**, *25*, 27–33. [CrossRef]

26. Sales, T.A.; Cardoso, M.D.G.; Guimarães, L.G.D.L.; Camargo, K.C.; Rezende, D.A.C.S.; Brandão, R.M.; Souza, R.V.; Ferreira, V.R.F.; Marques, A.E.; Magalhães, M.L.; et al. Essential Oils from the Leaves and Flowers of *Callistemon viminalis*: Chemical Characterization and Evaluation of the Insecticide and Antifungal Activities. *Am. J. Plant Sci.* **2017**, *8*, 2516–2529. [CrossRef]
27. Ndomo, A.F.; Tapondjou, L.A.; Ngamo, L.T.; Hance, T. Insecticidal Activities of Essential Oil of *Callistemon viminalis* applied as Fumigant and Powder against Two Bruchids. *J. Appl. Entomol.* **2010**, *134*, 333–341. [CrossRef]
28. Visakh, N.U.; Pathrose, B.; Chellappan, M.; Ranjith, M.T.; Sindhu, P.V.; Mathew, D. Chemical Characterisation, Insecticidal and Antioxidant Activities of Essential Oils from Four *Citrus* spp. Fruit Peel Waste. *Food Biosci.* **2022**, *50*, 102163. [CrossRef]
29. Visakh, N.U.; Pathrose, B.; Narayanankutty, A.; Alfarhan, A.; Ramesh, V. Utilization of Pomelo (*Citrus maxima*) Peel Waste into Bioactive Essential Oils: Chemical Composition and Insecticidal Properties. *Insects* **2022**, *13*, 480. [CrossRef] [PubMed]
30. Visakh, N.U.; Pathrose, B.; Chellappan, M.; Ranjith, M.T.; Sindhu, P.V.; Mathew, D. Extraction and Chemical Characterisation of Agro-waste from Turmeric Leaves as a Source of Bio active Essential Oils with Insecticidal and Antioxidant Activities. *Waste Manag.* **2023**, *169*, 1–10. [CrossRef]
31. Debbabi, H.; El Mokni, R.; Nardoni, S.; Chaieb, I.; Maggi, F.; Nzekoue, F.K.; Caprioli, G.; Hammami, S. Chemical Diversity and Biological Activities of Essential Oils from Native Populations of *Clinopodium menthifolium* Subsp. *Ascendens* (Jord.) Govaerts. *ESPR* **2021**, *28*, 13624–13633. [CrossRef]
32. Shukla, R.; Singh, P.; Prakash, B.; Kumar, A.; Mishra, P. Nawal Kishore Dubey Efficacy of Essential Oils of *Lippia alba* (Mill.) N.E. Brown and *Callistemon lanceolatus* (Sm.) Sweet and Their Major Constituents on Mortality, Oviposition and Feeding Behaviour of Pulse Beetle, *Callosobruchus chinensis* L. *Food Agric.* **2011**, *91*, 2277–2283. [CrossRef]
33. Gad, H.A.; Ayoub, I.M.; Wink, M. Phytochemical Profiling and Seasonal Variation of Essential Oils of Three Callistemon Species Cultivated in Egypt. *PLoS ONE* **2019**, *14*, e0219571. [CrossRef]
34. Misra, L.N.; Huq, F.; Ahmad, A.; Dixit, A.K. Chemical Composition of the Essential Oils of *Callistemon lanceolatus* DC and *C. polandii* F.M. Bailey. *J. Essent. Oil Res.* **1997**, *9*, 625–628. [CrossRef]
35. Shrestha, S.; Poudel, A.; Satyal, P.; Dosoky, N.; Chhetri, B.; Setzer, W.; Correspondence, W. Setzer Chemical Composition and Biological Activity of the Leaf Essential Oil of *Callistemon citrinus* from Nepal. *J. Essent.* **2015**, *3*, 29–33.
36. Chaaban, S.B.; Hamdi, S.H.; Mahjoubi, K.; Jemâa, J.M.B. Composition and Insecticidal Activity of Essential Oil from *Ruta graveolens*, *Mentha pulegium* and *Ocimum basilicum* against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *JPDP* **2019**, *126*, 237–246. [CrossRef]
37. Basaid, K.; Chebli, B.; Mayad, E.H.; Furze, J.N.; Bouharroud, R.; Krier, F.; Barakate, M.; Paulitz, T. Biological Activities of Essential Oils and Lipopeptides Applied to Control Plant Pests and Diseases: A Review. *Int. J. Pest Manag.* **2020**, *67*, 1–23. [CrossRef]
38. Brito, V.D.; Achimón, F.; Pizzolitto, R.P.; Ramírez Sánchez, A.; Gómez Torres, E.A.; Zygadlo, J.A.; Zunino, M.P. An Alternative to Reduce the Use of the Synthetic Insecticide against the Maize Weevil *Sitophilus zeamais* through the Synergistic Action of *Pimenta racemosa* and *Citrus sinensis* Essential Oils with Chlorpyrifos. *J. Pest Sci.* **2020**, *94*, 409–421. [CrossRef]
39. Abdelmalek, E.M.; Ramadan, M.A.; Darwish, F.M.; Assaf, M.H.; Mohamed, N.M.; Ross, S.A. Callistemon Genus—A Review on Phytochemistry and Biological Activities. *Med. Chem.* **2021**, *30*, 1031–1055. [CrossRef]
40. Singh, S.; Saini, M.K. Broad Spectrum Activity of Essential Oils in Managing Stored Grain Pests. *Indian J. Entomol.* **2022**, *83*, 854–864. [CrossRef]
41. Fall, R.; Ngom, S.; Sall, D.; Sembène, M.; Samb, A. Chemical Characterization of Essential Oil from the Leaves of *Callistemon viminalis* (D.R.) *Melaleuca leucadendron* (Linn.). *Asian Pac. J. Trop. Biomed.* **2017**, *7*, 347–351. [CrossRef]
42. Khani, M.; Marouf, A.; Amini, S.; Yazdani, D.; Farashiani, M.E.; Ahvazi, M.; Khalighi-Sigaroodi, F.; Hosseini-Gharalari, A. Efficacy of Three Herbal Essential Oils against Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Essent. Oil-Bear. Plants* **2017**, *20*, 937–950. [CrossRef]
43. Abdelgaleil, S.A.M.; Mohamed, M.I.E.; Shawir, M.S.; Abou-Taleb, H.K. Chemical Composition, Insecticidal and Biochemical Effects of Essential Oils of Different Plant Species from Northern Egypt on the Rice Weevil, *Sitophilus oryzae* L. *J. Pest Sci.* **2015**, *89*, 219–229. [CrossRef]
44. Danga, S.P.Y.; Nukenine, E.N.; Younoussa, L.; Adler, C.; Esimone, C.O. Efficacy of *Plectranthus glandulosus* (Lamiaceae) and *Callistemon rigidus* (Myrtaceae) Leaf Extract Fractions to *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J. Insect Sci.* **2015**, *15*, 139. [CrossRef]
45. Khan, M.M.; Siddiqui, J.A.; Ullah, R.M.K.; Raza, M.F. Effect of Different Biopesticides on Mortality and Their Synergetic Effect on the Fecundity of *Tribolium castaneum* (Herbst, 1797). *J. Biopestic.* **2020**, *13*, 142–152. [CrossRef]
46. Janaki, S.; Zandi-Sohani, N.; Ramezani, L.; Szumny, A. Chemical Composition and Insecticidal Efficacy of *Cyperus rotundus* Essential Oil against Three Stored Product Pests. *Int. Biodeterior. Biodegrad.* **2018**, *133*, 93–98. [CrossRef]
47. Devi, M.A.; Sahoo, D.; Singh, T.B.; Rajashekar, Y. Toxicity, Repellency and Chemical Composition of Essential Oils from Cymbopogon Species against Red Flour Beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *JCF* **2019**, *15*, 181–191. [CrossRef]
48. Hamzavi, F.; Moharramipour, S.; Talebi, A.A. Fumigant toxicity of *Eucalyptus camaldulensis* and *Callistemon viminalis* essential oils against *Tribolium confusum*. *Iran. J. Plant Prot. Sci.* **2011**, *42*, 241–249. [CrossRef]
49. Lee, B.-H.; Annis, P.C.; Tumaalii, F.; Choi, W.-S. Fumigant Toxicity of Essential Oils from the Myrtaceae Family and 1,8-Cineole against 3 Major Stored-Grain Insects. *J. Stored Prod. Res.* **2004**, *40*, 553–564. [CrossRef]

50. Liang, Y.; Li, J.L.; Xu, S.; Zhao, N.; Zhou, L.; Cheng, J.; Liu, Z.L. Evaluation of Repellency of Some Chinese Medicinal Herbs Essential Oils against *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **2013**, *106*, 513–519. [CrossRef]
51. Ebadollahi, A.; Taghinezhad, E.; Setzer, W.N.; Chen, G. Susceptibility of *Tribolium castaneum* (Coleoptera: Tenebrionidae) to the Fumigation of Two Essential *Satureja* Oils: Optimization and Modeling. *Processes* **2021**, *9*, 1243. [CrossRef]
52. Campolo, O.; Giunti, G.; Russo, A.; Palmeri, V.; Zappalà, L. Essential Oils in Stored Product Insect Pest Control. *J. Food Qual.* **2018**, *2018*, 6906105. [CrossRef]
53. da Silva Lunguinho, A.; das Graças Cardoso, M.; Ferreira, V.R.F.; Konig, I.F.M.; Gonçalves, R.R.P.; Brandão, R.M.; Caetano, A.R.S.; Nelson, D.L.; Remedio, R.N. Acaricidal and Repellent Activity of the Essential Oils of *Backhousia citriodora*, *Callistemon viminalis* and *Cinnamodendron dinisii* against *Rhipicephalus* spp. *Vet. Parasitol.* **2021**, *300*, 109594. [CrossRef] [PubMed]
54. Paranagama, P.A.; Gunasekera, J.J. The Efficacy of the Essential Oils of Sri Lankan *Cinnamomum zeylanicum* Fruit and *Micromelum minutum* Leaf against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Essent. Oil Res.* **2011**, *23*, 75–82. [CrossRef]
55. Visakh, N.U.; Pathrose, B.; Narayanankutty, A. Characterization of Secondary Metabolites from the Leaves of Curry Leaf (*Murraya koenigii* L.) Essential Oils with Insecticidal Activities against Stored Product Insects. *Biocatal. Agric. Biotechnol.* **2023**, *54*, 102973. [CrossRef]
56. Bali, A.S.; Batish, D.R.; Singh, H.P.; Kaur, S.; Kohli, R.K. Chemical Characterization and Phytotoxicity of Foliar Volatiles and Essential Oil of *Callistemon viminalis*. *J. Essent. Oil-Bear. Plants* **2017**, *20*, 535–545. [CrossRef]